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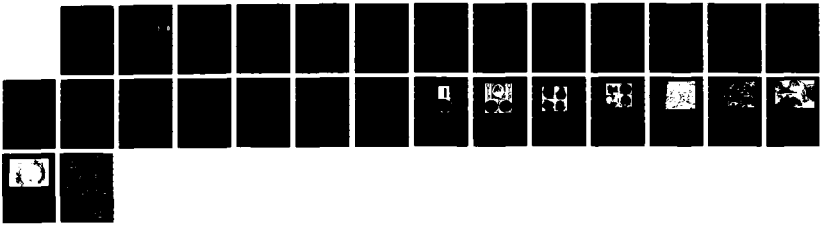

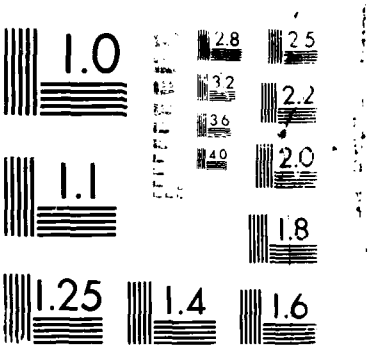
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AN EVALUATION OF TWO CONFIGURATIONS OF TRICALCIUM PHOSPHATE FOR TREATING CRANIOTOMIES

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An Evaluation of Two Configurations of Tricalcium Phosphate for Treating Craniotomies

Biodegradable beta-tricalcium phosphate disks (TCP) of 2 configurations were inserted into 15mm diameter craniotomy wounds and non-treated control sites were evaluated in 60 rabbits. There were no adverse tissue reactions and no apparent difference in the clinical appearance of the 12 and 24 week implanted disks. By 36 weeks and continuing to 48 weeks, the omnidirectional TCP (OTCP) implants were degrading more rapidly than the unidirectional TCP (UTCP) implants, with degradation progressing centripetally and replacement by woven bone and maturing lamellar bone. Host implant interface of both TCP configurations was a bone bond without interposed soft tissue. TCP disks may be clinically useful for craniotomy repair.

Key words: Bone regeneration, tricalcium phosphate disks, calvaria

TRICALCIUM PHOSPHATE FOR CRANIOTOMIES

Regeneration of bone of the facial skeleton lost as a consequence of trauma, disease, or resective surgery has been a goal sought by oral and maxillofacial surgeons. Although many materials have been used for osseous wound repair, no agent currently available provides the surgeon with a predictable level of bone regeneration. Autogenous grafts and allogeneic implants are the substances most commonly used by surgeons to treat bone defects; unfortunately, these materials have a failure rate ranging from 13-30 percent.[1] Ceramics and polymer alloplastic materials have been reviewed as possible alternatives to the traditionally used autogenous and allogeneic bone preparations.[2,3] To the best of our knowledge, there have been no studies that evaluated TCP in the form of disks for repairing non healing, 15 mm skull defects for a period of 48 weeks. It was the purpose of this study, therefore, to determine if disk forms of unidirectional and omnidirectional, biodegradable tricalcium phosphate (100% beta phase) could be used for treating craniotomies.

MATERIALS AND METHODS

The unidirectional tricalcium phosphate (UTCP) disks were prepared by an extrusion technique followed by laminating and rolling to produce single direction, 600 micrometer channels. A napthalene void former technique was used to manufacture the omnidirectional tricalcium phosphate

(OTCP) disks. These disks had non-interconnected, random pores measuring an average 100-150 micrometers in size. Both disk configurations consisted of sintered 100% beta phase TCP determined by x-ray diffraction spectra. Disk geometry was 15mm in diameter x 3.5mm in thickness. Implants were steam sterilized prior to insertion into the experimental animals. Sixty adult New Zealand white rabbits (skeletal maturity determined by radiographic closure of epiphyseal plates) of mixed sex, weighing 6-7 lbs. were divided equally into 3 treatment groups: UTCP (Figs. 1A and 1B), OTCP (Figs. 1C and 1D), and control (Fig. 1E). The anesthetic was a cocktail of ketamine HCl, USP (100mg/ml), xylazine, USP (100mg/ml), and sterile water (10:1:5, by volume) administered intramuscularly at a dose of 1cc/4 lbs. into the left hind leg. Prophylactic antibiotic coverage was obtained by using 300,000 units of benzathene penicillin G and procain penicillin G (Flocillin^{R}) in the right hind leg one hour before surgery. Calvarias were shaved, prepped, draped, and an incision was made in the midsaggital line of the skull. The skin and periosteum were reflected and a 15mm craniotomy was created in the parietal bones, using a trephine in a dental rotary handpiece with copious sterile saline irrigation. Circular UTCP or OTCP implants of identical size to the cranitomies were inserted securely into the bony wounds (Figs. 1F and 1G) and soft tissue was closed in layers with 3-0 Dexon^{TM} sutures. Each of the three treatment groups

was divided equally into four temporal groups of 12,24,36, and 48 weeks. At the appropriate time, each group was euthanatized with an overdose of sodium pentabarbital. The calvarias were exposed and the implant and control sites were removed with approximately 2-3mm of surrounding host bone using a number 703 dental bur and copious saline irrigation. Specimens were radiographed using a Minishot II Cabinet x-ray System (TFI Corporation) (3 milliamps, 15 Kvp, 20 secs) with Kodak X-Omat TL x-ray film. Specimens then were placed immediately into 70 percent ethanol for processing (unstained and undecalcified) in polymethyl methacrylate and sectioning to 75-100 micometers using a Buehler™ isomet saw. The thick sections were attached to glass slides and were evaluated histologically and histomorphometrically using a Zeiss Universal Microscope and Videoplan™ Image Analysis System. The percent of remaining UTCP and OTCP across the 15mm craniotomies and the area of bony fill in the 3 treatment groups were determined by random measurements of 6 fields from bony margin to bony margin. Between group comparisons of the remaining TCP and area of osseous fill at each time period were made using a student's t-test for unpaired data.

RESULTS

Twelve Weeks

Clinical findings revealed virtually no changes at the OTCP or UTCP periphery. Controls displayed fibrous union

across the 15mm craniotomy without evidence of bone repair. Implants appeared tissue tolerant. Histologically and histomorphometrically, there was minimal peripheral ingrowth of woven bone into the UTCP channels and into the randomly oriented pores of the OTCP (Tables 1 and 2). Radiographically, the impression was similar to the clinical and histological findings (Figs. 2A and 2B).

Twenty-Four Weeks

The appearance of both configurations of implants was similar to the 12-week groups. There was a suggestion of more peripheral decay of material in the OTCP than in the UTCP; however, clinical assessment was equivocal. Histologically, OTCP demonstrated obvious peripheral degredation with numerous peninsulas of woven bone invading the degrading implant. In contrast, the UTCP displayed frank ingress of woven bone into channels and minimal peripheral implant decay. Histomorphometrically, there were no statistically significant differences between treatments (Table 1 and 2). Radiographically, there was an irregular bony margin in the OTCP implants (Fig. 2C); whereas, the UTCP disks appeared to be more structurally intact (Fig. 2D). Control craniotomies appeared to be healed exclusively by a soft, fibrous tissue; however, two of the five sites displayed some irregularly shaped radiopacities.

Thirty-six Weeks

Clinically, both OTCP and UTCP appeared to be intact.

Palpation of the necropsy specimens revealed a firm, hard material without evidence of adverse tissue reaction. Histologically, a sequence of events paralleling the 24-week specimens was apparent, with the OTCP disks demonstrating considerably more peripheral disintegration than the UTCP disks. In the OTCP implants, woven bone was deposited directly on and surrounded OTCP fragments. The OTCP host bone margin displayed no fibrous interface, but rather, a direct implant to bone type of bonding. The UTCP showed histological evidence of peripheral degradation and channel invasion with woven bone; however, the extent of new bone formation was substantially less than with the OTCP implants due to the slower breakdown of UTCP (Tables 1 and 2). Radiographically, the appearance of the necropsy specimens confirmed the clinical and histological pictures (Figs. 3A and 3B).

Fourty-Eight Weeks

Clinically, two of the control sites appeared to have bony islands embedded within healing fibrous tissue. Both implant configurations were hard and firmly fixed to the host margins without indication of adverse tissue reaction. Histologically and histomorphometrically, approximately 90 percent of the OTCP implant disks had degraded and the OTCP was replaced with woven bone and maturing lamellar bone. Centrally, there was evidence of OTCP. The UTCP was breaking down peripherally; however, approximately 80 percent of the material was intact. Within the channels there was woven bone

and some lamellar bone. Significant differences existed between treatment groups (Tables 1 and 2). Radiographic assessment paralleled the histological and clinical observations (Figs. 3C and 3D).

DISCUSSION

Ceramics, especially the biodegradable beta-tricalcium phosphates, have properties which may enable them to serve as alternatives to bone grafts and implants in selected situations.[4] Porous tricalcium phosphate may function as a scaffold that can be scavenged, incorporated, or converted chemically into host bone.[5] Despite the poor physical property of strength, the degradable ceramics (i.e., TCP) may find utility as a bone repair substitute in low load and relatively low stress bearing areas, such as flat bones of the calvaria and mid-face.[3,4]

An important attribute of the TCP bone substitutes is the pore configuration that allows for osteoconduction. Studies by Klawitter and Hulbert and Nade et al., reported that a minimal pore size of about 100 micrometers was the optimum for new bone growth into porous ceramics.[6,7] The average pore size of the OTCP implant disks that we used was 100-150 micrometers; however, many pores were 25 micrometers and some were greater than 200 micrometers. Furthermore, pores were not inter-connected; many blind alleys existed.

The UTCP, on the other hand, consisted of channels throughout the length of the implant disk that were approximately 600 micrometers in diameter and the porosity that existed was not greater than 15-20 micrometers. The pore design of the OTCP implant disks might explain why they degraded more rapidly than their UTCP counterparts (Figs. 4 and 5). de Groot maintains that the rate of biodegredation of TCP is determined by microporosity.[8] The OTCP disks were more porous and had more surface area than did the UTCP disks. Surface area is a major factor in determining dissolution rates of solid material.[2] In concert with the more rapid biodegration of OTCP, there was significantly more new bone that developed across the OTCP implants than the UTCP implants (Fig. 6). Untreated controls did not heal by bony union over the duration of the study (Fig. 7). This fact demonstrates that a 15mm diameter craniotomy in a skeletally mature rabbit is a critical size defect appropriate for testing bone repair materials.[9]

Metsger, Driskell, and Paulrud reviewed the resorbable tricalcium phosphate ceramics for dental applications.[10] They concluded that the tissue compatibility and osseous repair using TCP appeared to be superior to other synthetic materials. Moreover, they stated that there were no reports of adverse reactions attributed to TCP. A recent study by Klein et al. investigated 100% beta phase TCP (beta-whitlockite) in the form of micropore or macropore

cylinders (2.2mm diameter x 2.5mm length) to repair bone defects in rabbits' tibiae.[11] The authors determined that microporosity plays an important role in the biodegradation rate of beta-phase ceramics. (Microporosity was not defined; however, the authors described macroporosity as being 200-500 micrometers.) Interestingly, Klein et al. determined that the resorbing TCP evoked an inflammatory response at the implant site (up to 16 months post-insertion) that was characterized by lymphocytes, plasma cells, and phagocytic cells.[11] Moreover, subiliac lymph nodes were reported to contain macrophages with engulfed ceramic particles. To the best of our knowledge, the Klein et al. study is unique in regards to adverse tissue reactions to pure beta phase TCP. We did not encounter inflammatory problems elicited from the TCP. We did not examine any lymph nodes to determine if ceramic particles had been phagocytosed and deposited in regional nodes.

A study by Uchida et al. evaluated three types of ceramics (calcium aluminate, calcium hydroxypatite, and tricalcium phosphate) in the form of porous disks (3mm diameter x 1mm thick, with pore sizes of either 150-210 micrometers or 210-300 micrometers).[4] Disks were placed into defects in parietal bones of Sprague-Dawley rats and New Zealand white rabbits. Defects only involved the outer table. The authors reported that for all ceramics a foreign body or inflammatory cellular response to the implants was minimal and a few giant cells were noted within ceramic pores. No

bone growth was observed in any of the pores of the TCP disks. Unfortunately, the authors did not present any data demonstrating degradation of the TCP over the 128 day course of their experiment. In our study, the residual OTCP and UTCP at 24 weeks was 78% and 84%, respectively. However, by 36 weeks there was a significant decrease in OTCP disk material, with only 43% remaining. In contrast, the UTCP disks were still present to 80% of their original structure. Furthermore, contrary to Uchida et al., we did observe new woven bone ingrowth in pores and channels of OTCP and UTCP (Fig. 6). Other authors have reported similar bone osteoconduction.[2,5-7]

The biodegradable ceramics have a narrow range of application in osseous repair. Because of their brittleness, they should not be used in weight bearing, high stress areas. In low load bearing locations such as the flat bones of the craniofacial complex or in alveolar clefts, pure beta-tricalcium phosphate may find utility. However, because TCP is not osteoinductive and because of the paucity of bone marrow interposed between flat bone diploe, an agent is needed to suppliment the osteoconductive affect of TCP. Urist, Lietze, and Dawson incorporated bone morphogenetic protein (BMP) into porous TCP and implanted it into quadriceps muscles in rats.[12] They determined that 12 times as much bone was developed from this composite in contrast to similarly treated sites using only BMP. The bone inductive

capacity of the BMP may possibly be exploited by using it in combination with OTCP implants for treating bony deficiencies of the cranio-maxillofacial complex.[13]

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LEGENDS

Figs. 1A and 1B: Side and top views of UTCP disks.

Figs. 1C and 1D: Side and top views of OTCP disks.

Figs. 1E-G: An untreated control craniotomy (15mm, O.D.) (E) and 2 configurations of disks (UTCP-F and OTCP-G) following insertion into rabbits.

Fig. 2: Radiograph of OTCP (A) and UTCP (B) at 12 weeks. At 24 weeks both TCP configurations appear to be radiographically intact (OTCP-C, UTCP-D).

Fig. 3: Radiographic appearance of OTCP (A) and UTCP (B) at 36 weeks confirms the histological view. At 48 weeks UTCP (D) is still evident radiographically; however, OTCP (C) seems to be almost totally replaced by new bone formation.

Fig. 4: OTCP with its 100-150 micrometer pore design at 12 weeks showing a host implant interface of soft tissue and bone. (2.5X. Unstained. OTCP: Black - right part of field; bony trabeculae of host margin - left part of field.)

Fig. 5: OTCP at 36 weeks with arrows defining host-implant interface. Numerous areas throughout degrading implant display a typical 36 and 48 week pattern of bony trabecular investment of the OTCP (Black). Pore form is no longer recognizable due to the degradation (2.5X. Unstained).

Fig. 6: A typically appearing UTCP disk at 36 weeks with a 600 micrometer channel (defined by black triangles) demonstrating some bony ingrowth. Polarized light was used and new bone and host bone appear white (2.5X. Unstained. Polarized).

Fig. 7: A radiograph of an untreated control craniotomy that did not heal by bony union(48 weeks).

TABLE 1

MEAN AREA OF BONE FILL

(mm^2)

TREATMENT	OTCP	UTCP	CONTROL (C)	p
TIME (WEEKS)				
12	2±0.5	4±1.0	5±2.0	NS
24	18±3	13±2	18±4	NS
36	82±6	21±2	23±3	*
48	123±11	29±5	49±6	**

RESULTS EXPRESSED AS MEANS OF 30 MEASUREMENTS (5 animals x 6 fields)±SEM

NS = NOT SIGNIFICANTLY DIFFERENT

* OTCP: SIGNIFICANTLY DIFFERENT THAN UTCP OR C; WHEREAS UTCP AND C DO NOT DIFFER ($p<0.05$)

** OTCP: SIGNIFICANTLY DIFFERENT THAN UTCP OR C ($p<0.0001$); UTCP DIFFERS FROM C ($p<0.05$)

TABLE 2

MEAN PERCENT REMAINING TCP

TYPE OF TCP	OMNIDIRECTIONAL (OTCP)	UNIDIRECTIONAL (UTCP)	p
TIME(WEEKS)			
12	89±6	91±5	NS
24	78±5	84±6	NS
36	43±3	80±7	<0.05
48	9±1	78±5	<0.05>0.0001

RESULTS EXPRESSED AS MEANS OF 30 MEASUREMENTS

(5 animals x 6 fields)±SEM

NS = NOT SIGNIFICANTLY DIFFERENT

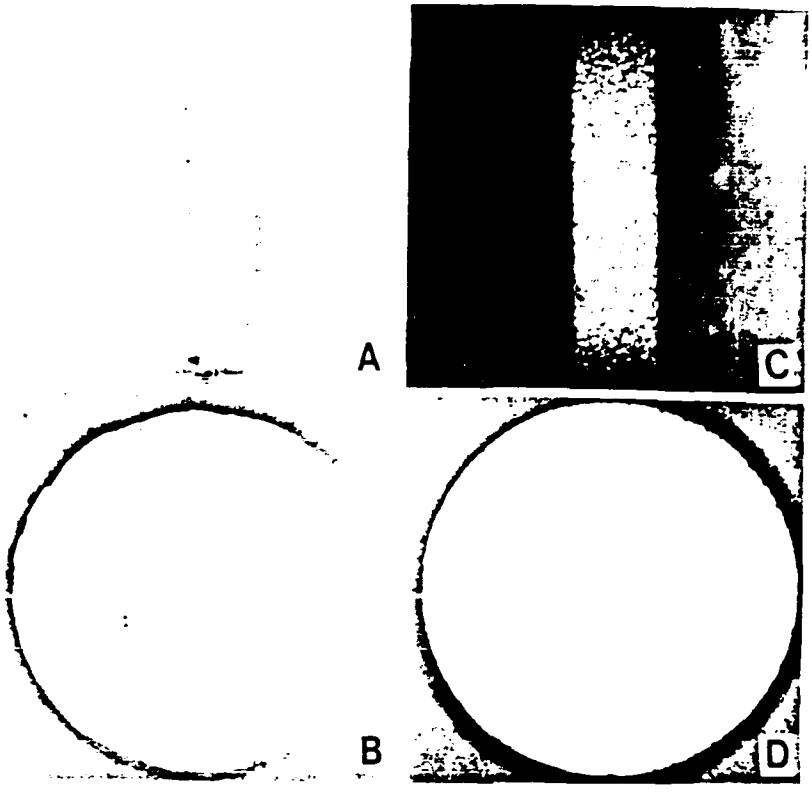

FIGURE 1A-D

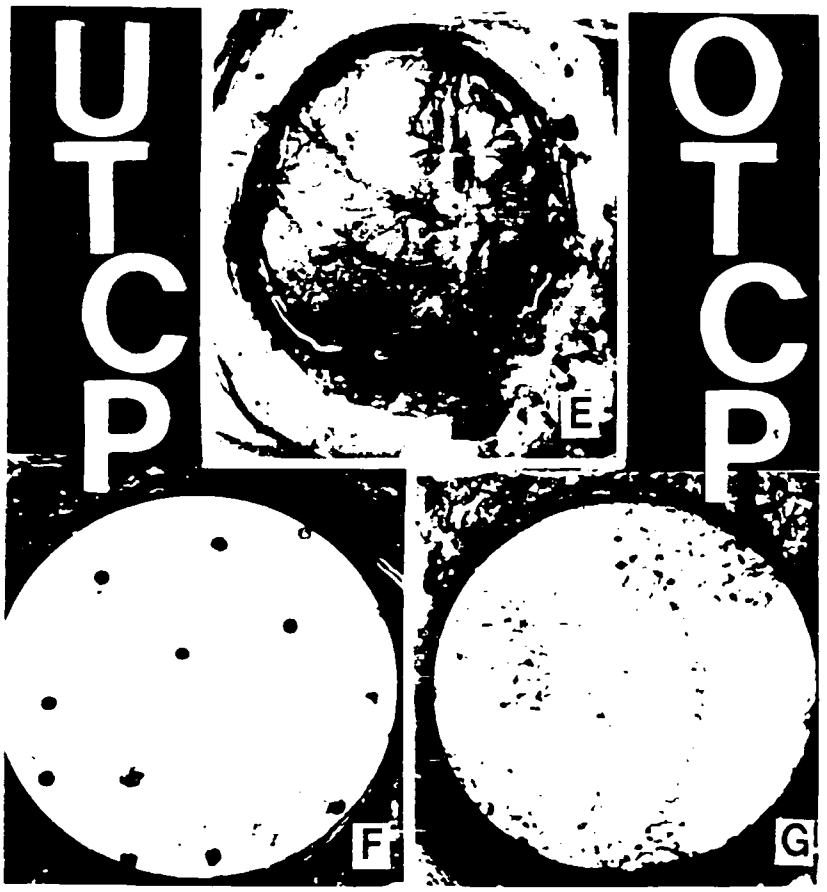

FIGURE 1E-G

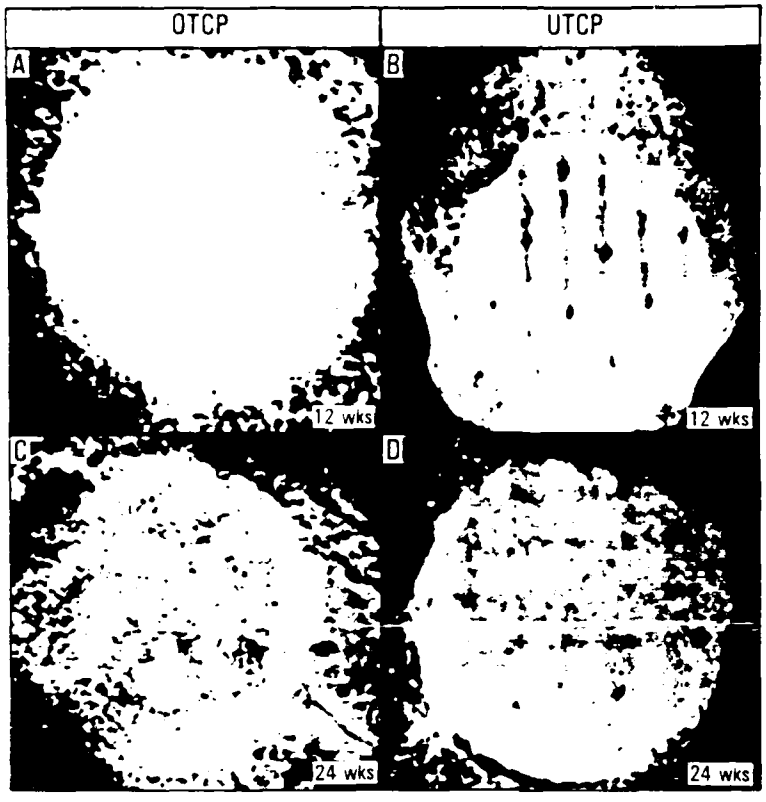

FIGURE 2

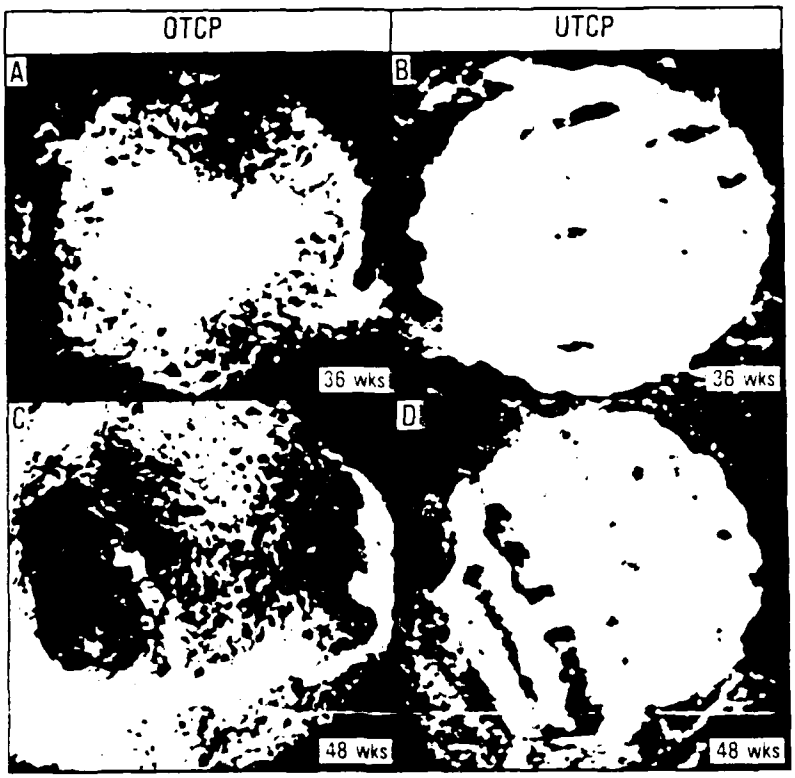

FIGURE 3

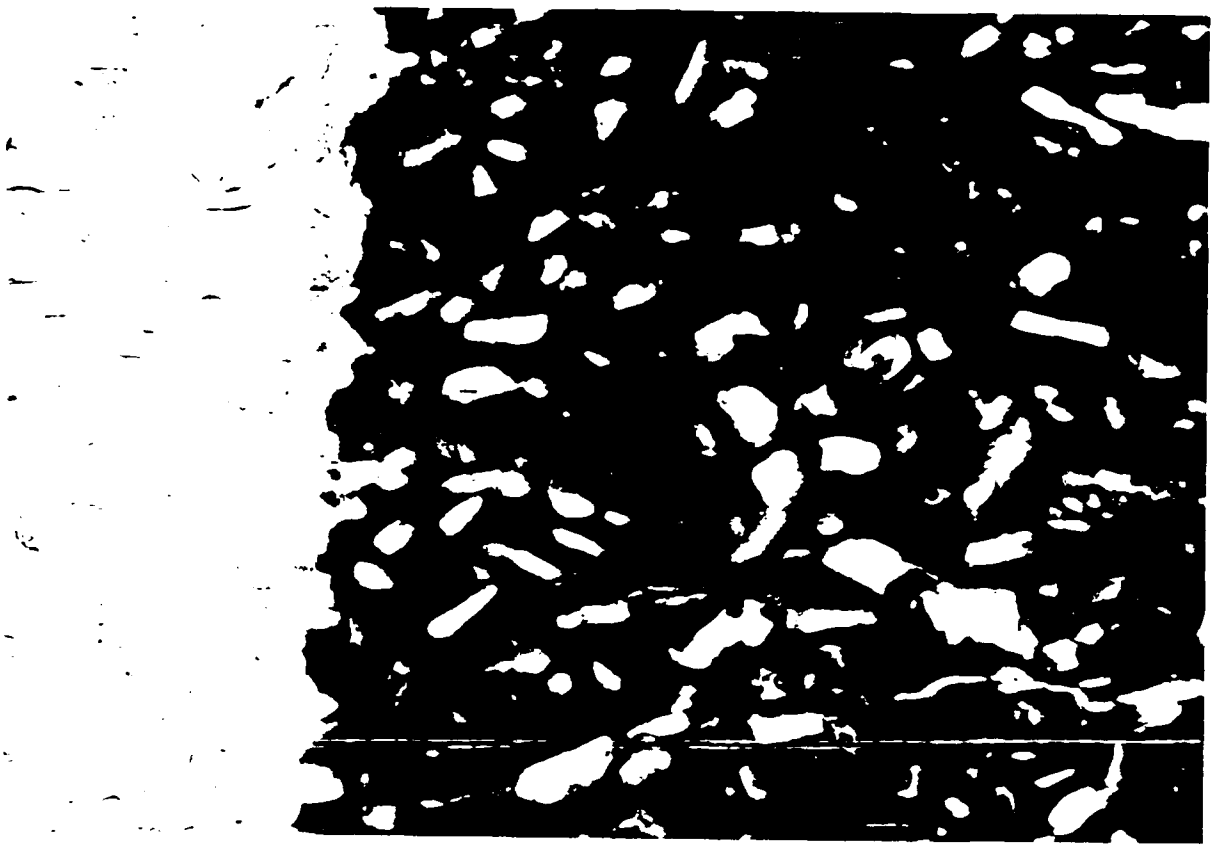

FIGURE 4

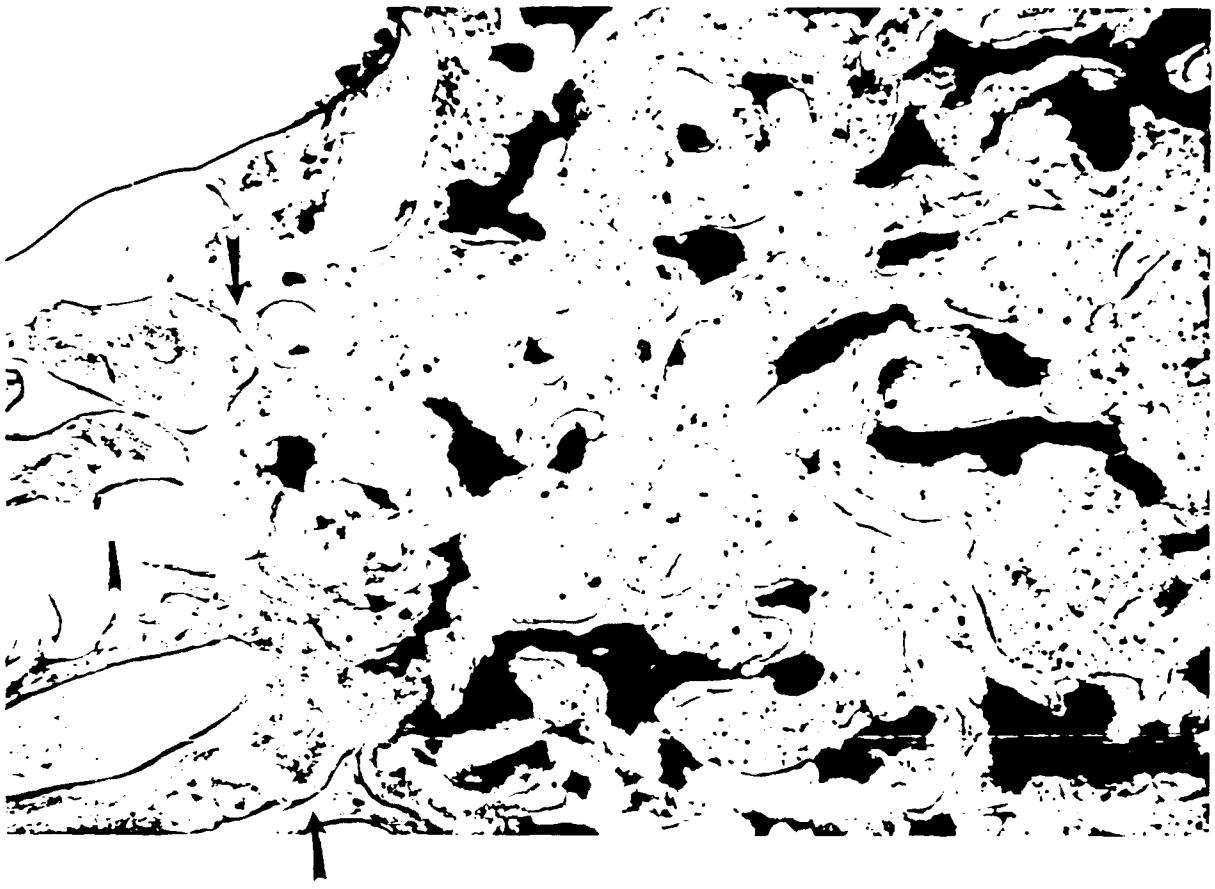

FIGURE 5

FIGURE 6

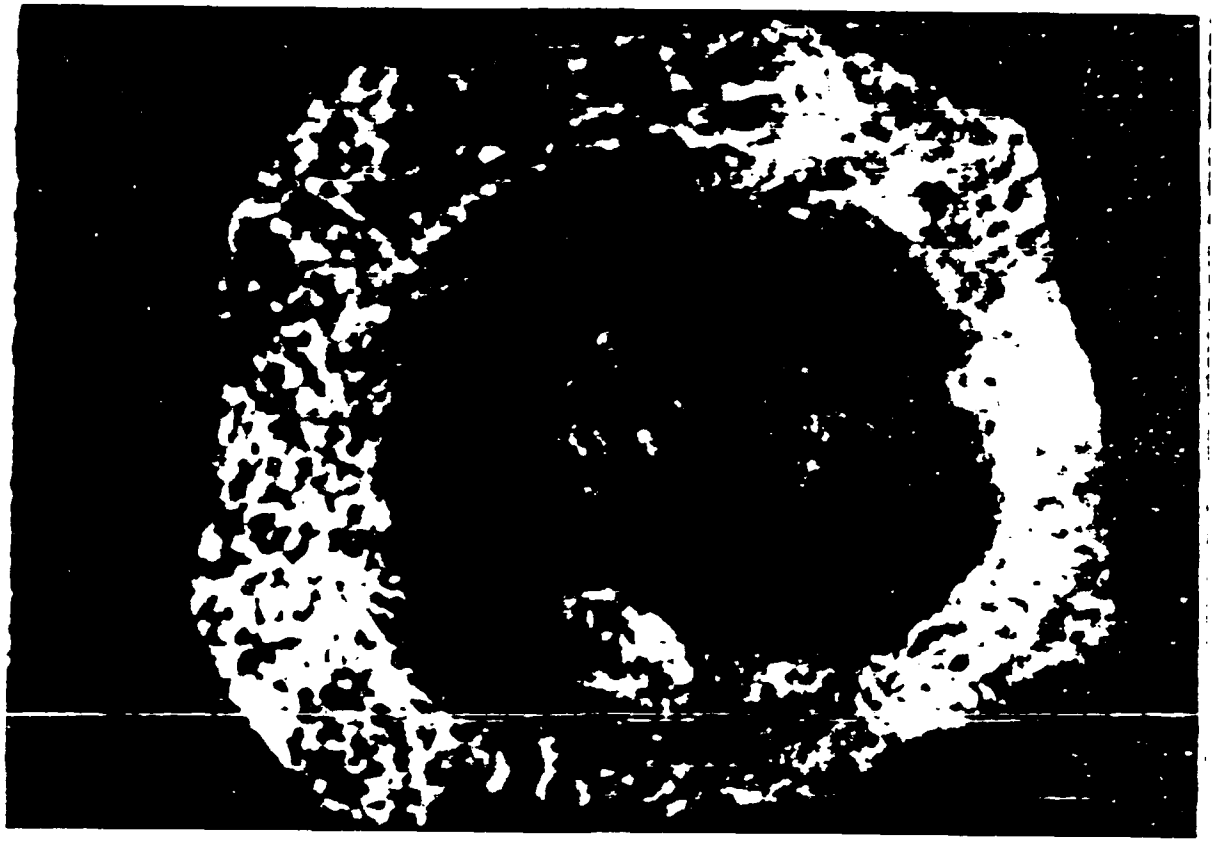

FIGURE 7

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